

A rapid procedure for preparation of radulae for routine research with the Scanning Electron Microscope

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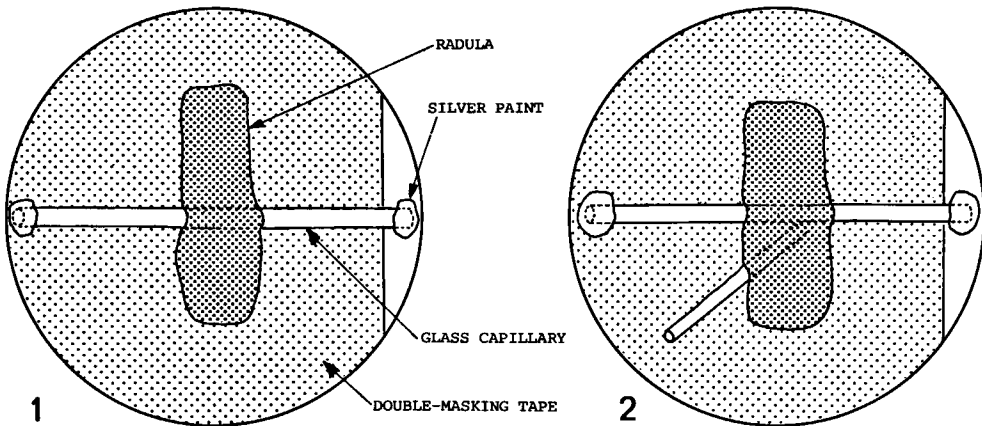
About ten years ago the first publications appeared on the use of the Scanning Electron Microscope (hereafter abbreviated SEM) for research on radulae (Runham & Thornton, 1967; Thompson & Hinton, 1968). This instrument has now proved to be an eminent tool and the method as such is becoming more and more a routine procedure for many malacologists.

Prior to viewing the specimens with a SEM, the preparation procedure is an essential step. Data on this procedure have been given briefly in most publications dealing with the SEM-method for radulae. The only survey of methods of preparation has been given by Solem (1972).

As to the procedure to be followed during preparation of a specimen for the SEM we feel that one should distinguish between two types of research, viz., specialized and routine research. With the first type we denote the research on the structure and functioning of the radula, which is therefore the principal object of the research. In our opinion preparation techniques for these specialized investigations should be as perfect as possible and critical point-drying or freeze-drying should be recommended when working with the SEM.

A second type of research on radulae is, e.g., during the taxonomic revision of a group. Then the study of radulae may be of secondary importance and a rapid procedure is required to study and compare large series of specimens. Hence time-consuming methods such as critical point-drying and freeze-drying need not be employed. Moreover, personal experience with freeze-drying has proved to yield no significantly better results during routine research (cf. Solem & Van Goethem, 1974).

The preparation procedure as described by us hereafter was elaborated during our research on Bulimulidae, which are small to medium-sized land snails. Our experience



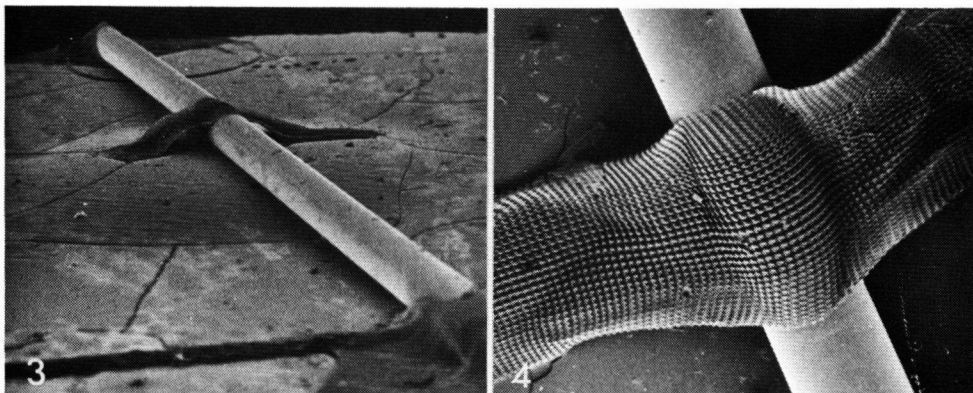
Figs. 1-2. Mounting of the radula. The position shown in fig. 2 only applies to larger radulae.

indicates that the radula should be at least two mm long to obtain good results. Smaller radulae may be treated with the 'splinter-method', also described below.

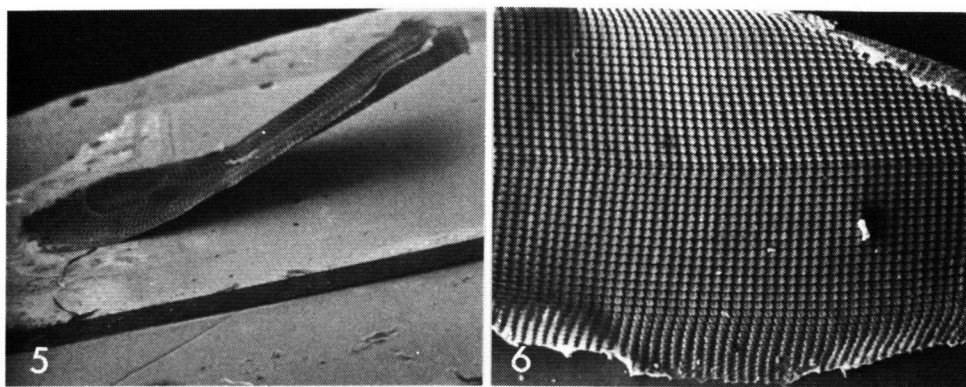
The second author initiated his research on radulae using the SEM procedure as outlined by Solem (1972). As the use of rubber cement was not completely satisfactory he switched to a method worked out by Dr. E. Gittenberger. In this method a splinter of a cover-glass is placed on a slide and covered with 70% ethyl alcohol. The dissected radula is positioned on the splinter with the teeth upwards in such a way that the greater part of the radula is on the splinter. Once in the desired position the radula is covered by an intact cover-glass, which is made heavier to obtain eventually a flat radula. The ethyl alcohol is drained with the aid of pieces of filter-paper and replaced by 96% ethyl alcohol, which is allowed to evaporate. The cover-glass may then be removed and the radula will be found to adhere to the splinter, which can be mounted on the SEM-stub. However, the results obtained by this method have not always been satisfactory either. Often the radula was found to adhere not only to the splinter but also to the underlying slide. In other cases the radulae were found to adhere to the top cover-glass instead of to the splinter. Moreover by placing weights on the cover-glass the radula may shift position, often with damage to the latter, and the evaporation rate of the ethyl alcohol is very low. Finally, during this method the radula does not always bend, fold, etc. as liked.

The following procedure has evolved from our efforts to improve the existing methods and to adapt them to our type of research.

The buccal mass is macerated in a ca. 10% potassium hydroxide (KOH)-solution. By placing the vial in an incubator at 60°C this process is fairly well completed within 10 to 20 minutes. The radula can now easily be dissected from the soft tissue of the buccal mass. When the radula is completely free from any tissue it is placed in a vial containing



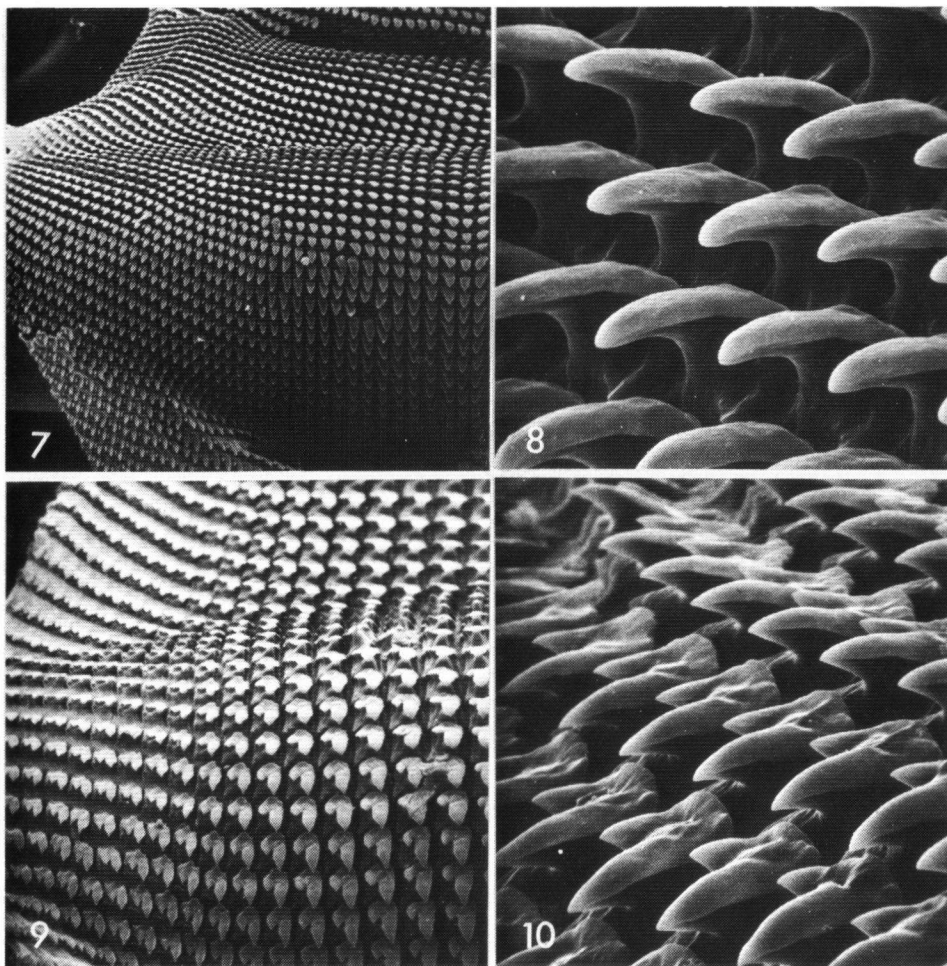
Figs. 3-4. General views of radula mounted via the procedure outlined in this paper (*Bostryx bermudezae* Weyrauch, 16 x and 40x respectively).



Figs. 5-6. Radulae mounted via the 'splinter-method' [5: *Bothriembryon gunni* (Sowerby), 18x [N.B. Radulae normally not so uplifted as shown here]; 6: *Auris bilabiata* (Broderip & Sowerby), 16x].

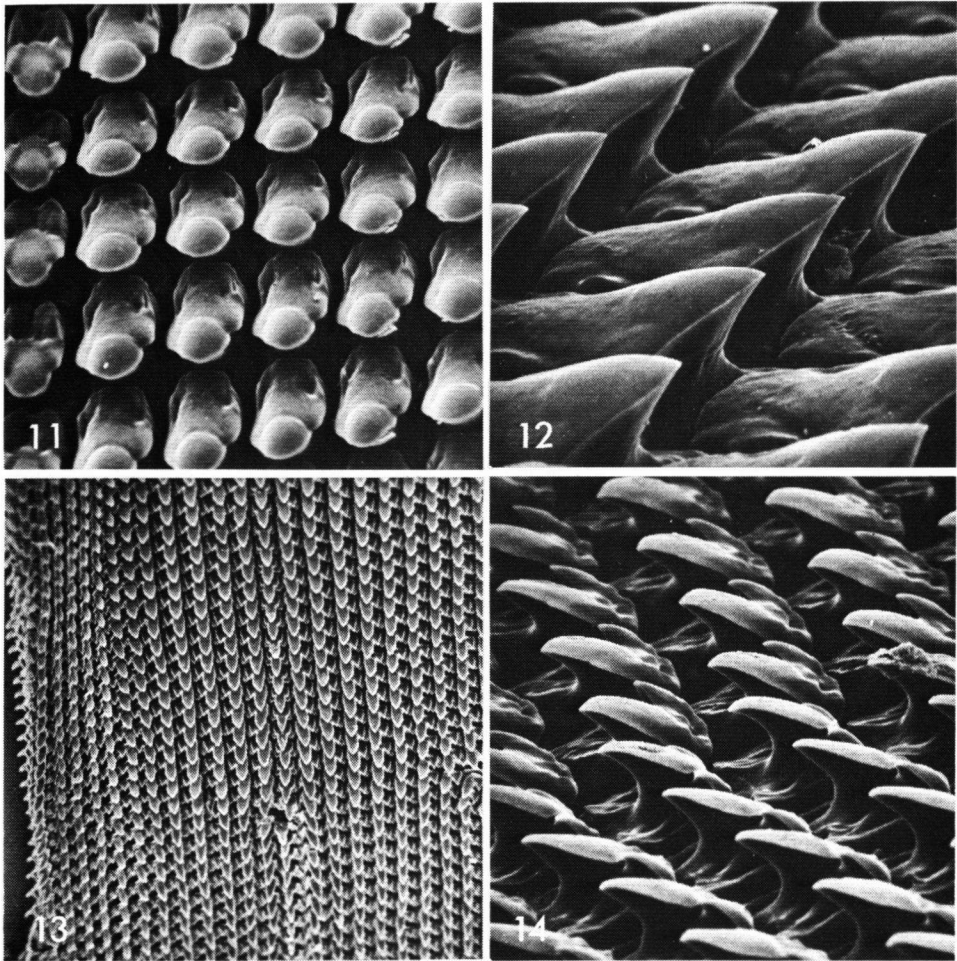
70% ethyl alcohol and for cleaning purposes is given an ultrasonic treatment of 5-15 seconds.

Meanwhile a SEM-stub has been covered for about 80% with double masking tape and a drop of silver paint is placed on the transition from tape to stub to secure the discharge of the electric current from the radula. A thin glass capillary (outer diameter 0.2-0.5 mm) is glued on to the stub with silver paint. The stub is covered with a drop of 70% ethyl alcohol and the radula may now be transferred from the vial to the stub, which is placed



Figs. 7-10. Some results obtained with the procedure outlined in this paper [7-8: *Bostryx rhodolarynx apurimacensis* (Dall), general view and interaction of transverse rows, 71x and 715x respectively; 9-10: *Bostryx bermudezae* Weyrauch, idem, 162x and 780x respectively].

under a dissection microscope. At a magnification of 100x it can usually be determined which side of the radula bears the denticles (cf. also Meeuse, 1950: fig. 1), after which the radula is positioned over the glass capillary in such a way that the capillary is perpendicular to the longitudinal axis of the radula. This will result in erection of the teeth which are on top of the capillary, thus simulating a feeding position. See figs. 1, 3-4 and compare



Figs. 11-14. Some results obtained with the 'splinter-method' [11-12: *Auris bilabiata* (Broderip & Sowerby), general view and interaction of transverse rows, 160x and 375x respectively; 13-14: *Geoceras columellaris* (Reeve), idem, 160x and 845x respectively].

figs. 5-6. The interaction of the transverse rows may now be studied e.g., by viewing from the right or left side at an angle of 45 degrees. Another possibility is to position the radula with its longitudinal axis over the glass capillary, or diagonally. This will give additional information (cf. Solem, 1972). When working with larger radulae these orientations may be combined by using two glass capillaries which are placed at an angle

of e.g., 45 degrees (fig. 2). For smaller radulae we recommend to use a specimen for each different position.

Once the radula has its final position two additional capillaries are placed on the radula parallel to the first capillary in order to keep the radula in the desired position. The alcohol is now allowed to evaporate completely. We have obtained good results both by evaporation in the open air at room temperature as well as by placing the stub in an incubator at 37°C. Because the radula is entirely exposed to the air the process of evaporation is relatively rapid.

The radulae may now be stored in any snap top box before viewing in the SEM. Coated specimens may also be stored in these boxes, although Solem (1972: 331) recommends storage in a vacuum desiccator when unfavorable atmospheric conditions prevail.

The results obtained with this method are shown in figs. 7-10, while figs. 11-14 give some results of the 'splinter-method'. The advantages of our method are: (1) a rapid procedure, therefore especially suitable for routine research of the radula (after dissection and cleaning it takes ca. 10-15 minutes to complete the process of mounting, whereas ca. 30-45 minutes are needed when using the 'splinter-method'); (2) the final result is obtained relatively easily and with great certainty.

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